

Review

# Nasal polyposis: insights in epithelial-mesenchymal transition and differentiation of polyp mesenchymal stem cells

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**Abstract:** Chronic rhinosinusitis is a common inflammatory disease of paranasal sinuses, which causes rhinorrhea, nasal congestion and hyposmia. The genetic predisposition or the exposure to irritants can sustain the inflammatory response and the development of nasal polyposis. Nasal polyps are benign and teardrop-shaped growths that project in the nasal cavities and originate from the ethmoid sinuses. This inflammatory process is associated with high expression of IL-5 cytokine and infiltration of eosinophils. Humanized monoclonal antibodies targeting IL-5 or its receptor, represent a therapeutic strategy in the treatment of nasal polyposis in combination with corticosteroids. The molecular pathogenesis of nasal polyps in CRS patients is associated to the epithelial-mesenchymal transition (EMT), a process in which epithelial cells lose their typical phenotype acquiring a mesenchymal phenotype. TGF $\beta$ /SMAD, ERK, and Wnt/ $\beta$ -catenin pathways are altered in EMT during the nasal tissue remodeling. miRNA and inhibitor molecules targeting these altered signaling pathways are able to interfere with EMT; which could lead to alternative therapies. Nasal polyps are an alternative source of mesenchymal stem cells which can be easily isolated from surgical biopsies. A molecular understanding of the biology of PO-MSCs will contribute to delineating inflammatory process underlying the development of nasal polyps.

**Keywords:** Chronic rhinosinusitis (CR), inflammation, nasal polyps, epithelial to mesenchymal transition (EMT), Polyp derived mesenchymal stem cells (PO-MSCs).

## 1. Introduction

Chronic rhinosinusitis (CRS) is a common multifactorial inflammatory disorder characterized by the inflammation of the paranasal sinuses and nasal cavity [1,2]. CRS patients can be classified into two groups according to nasal endoscopy properties: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP), which show an heterogeneous inflammatory patterns [3]. Nasal polyps are non-cancerous and painless growths originating from the ethmoid sinuses and affecting the nasal mucosa and paranasal sinuses. The etiology of polyps formation is not yet fully understood [4-6]. Numerous factors including anatomic disorders, genetic factors, infections caused by viruses, bacteria, fungi, as well as asthma, allergic rhinitis, non-allergic inhalants are associated with nasal polyp development and progression [7].

The nasal polyp formation involves a sequence of specific histological features: the mucosal epithelial rupture, proliferation of fibrous tissue through the injured epithelial, extracellular matrix (ECM) accumulation with edema and the proliferation of a granular tissue comprising thin-walled

vessels and inflammatory cell infiltration [8,9]. The inflamed nasal mucosa takes on a gelatinous texture, resembling grape like clusters, with a translucent and pale appearance, giving rise to nasal polyps [10].

The inflammatory process plays an important role in the pathogenesis of nasal polyposis. The different forms of CRS appear to be caused by inflammatory changes in the sinonasal mucosa. A Th2-mediated inflammatory process is usually found in CRSwNP, whereas both Th2- and Th1-mediated processes are found in CRSsNP [4]. CRSwNP can have different inflammatory profiles depending on related diseases, such as bronchial asthma, cystic fibrosis (CF), or NASID-Exacerbated Respiratory Disease (N-ERD). The inflammatory process is characterized by a multitude of cytokines and interleukins (IL) in the different cell types, importantly IL-4, IL-5 and IL-13 which are produced by Th2 cells. IL-4 is able to promote the differentiation of CD4<sup>+</sup> T cells into Th2 cells and at the same time inhibit interferon (IFN)- $\gamma$  production and Th1 response. The TH cytokine profile can vary considerably in different CRS patients from Europe, Asia and Australia [11].

Nasal polyp tissue displays robust levels of IL-5 compared to healthy controls suggesting that this cytokine is a key regulatory factor for eosinophil survival and activity. IL-5 produced by Type 2 T helpers (Th2) [12] as well as type 2 innate helper lymphoid cells (ILC2) [13], induces proliferation and maturation of eosinophils and is essential for their mobilization. Subsequently eosinophils migrate and accumulate into nasal tissues where they synthesize and release lipid mediators and enzymes causing edema and tissue damage respectively [14]. IL13 functions as an effector molecule that mediates eosinophilic inflammation, airway hyperresponsiveness and mucus hypersecretion [12]. In addition, two proteins of eosinophil degranulation, eosinophilic cationic protein (ECP) and the epithelium-derived neurotoxin (EDN) have been identified as effectors of epithelial damage and correlated with the number of activated eosinophils in circulation [15,16].

The revelation of the immunological mechanisms underlying chronic rhinosinusitis with nasal polyps has resulted in the production of monoclonal antibodies targeting the interleukin-5 pathway as an additional treatment to corticosteroids [17]. The effect of IL-5 could be antagonized by two types of humanized monoclonal antibodies targeting IL-5 (Reslizumab and Mepolizumab) or its receptor respectively (Benralizumab) [18]. Both kinds of antibodies are able to inhibit IL-5 signaling and to induce apoptosis of target cells via antibody-dependent cell-mediated cytotoxicity [19]. The consequence of this is a significant reduction in eosinophil counts in peripheral blood in humans [20]. A Double-Blind randomized trial showed the role of Mepolizumab in reducing the number of patients with CRSwNP, associated to recurrent nasal polyposis, needing surgery compared to the patients treated with placebo [21]. Humanized antibodies for IL-5 blocking the pathway can be considered a novel and effective therapeutic strategy, based on a molecular approach, for the treatment of recurrent CRSwNP with peripheral eosinophilia.

In this review, we discuss the current knowledge of the molecular mechanisms underlying the development of nasal polyps with particular regard to novel regulatory mechanisms involved in EMT transition. The understanding of the different cell signal transduction pathways involved in polyp pathogenesis will give the possibility to identify novel molecular-targeted agents that could be used to complement current therapeutic strategies. It is also highlighted that nasal polyps are an alternative source of mesenchymal stem cells with a potential in regenerative medicine. Polyp-derived mesenchymal stem cells (PO-MSCs) are a useful *in vitro* model for studying the immune modulatory properties in the nasal polyp microenvironment.

### **1.1 Role of Epithelial Mesenchymal Transition (EMT) in Chronic Rhinosinusitis with nasal polyp development: potential molecular strategies targeting EMT-related modulators.**

Healthy nasal epithelium consists of four cell types: basal cells, goblet cells, ciliated, and non ciliated columnar cells [22]. Basal cells have been identified as stem/progenitor cells able to self-renew and differentiate into other epithelial cell types [23]. Stem/progenitor cells have a central role in tissue homeostasis, repair, and regeneration of mucous membrane including the nasal mucosa [24]. The cellular pathogenesis of nasal polyps is related to a homeostatic imbalance between

the reduction in proliferation of nasal epithelial stem/progenitor cells [25], and the presence and differentiation of mesenchymal stem/progenitor cells (MSCs) [26].

EMT is a complex cellular process by which, epithelial cells lose their epithelial phenotype and acquire a mesenchymal one, following a chronic stimulus [27,28]. During EMT, on the one hand, epithelial markers, for example E-cadherin, are downregulated by several inducers of EMT acting as transcription factors such as Snail, Slug, Twist and Zeb; on the other hand, an upregulation of mesenchymal markers such as N-cadherin, alpha-smooth muscle actin ( $\alpha$ -SMA), vimentin and fibronectin, as well as matrix metalloproteinases (MMP) occurs [29]. This EMT process results in a weakening of cell-to-cell contacts and increase of motility.

### **1.2 TGF- $\beta$ 1 signaling is involved in the EMT process during CRSwNP pathogenesis.**

TGF- $\beta$ 1 signaling dysregulation was found in inflammatory polyps where it participates to sustain the characteristic remodeling of nasal mucosa [30]. TGF- $\beta$ 1 down-regulation is typically associated with CRSwNP, whereas TGF- $\beta$ 1 up-regulation is characteristic of CRSsNP [31]. TGF- $\beta$ 1 signaling acts as a potent driver in EMT, during nasal polyp formation and growth, inducing a loss of epithelial and gain of mesenchymal markers [32]. In addition, the TGF- $\beta$ 1 pathway activation increased the expression of endoplasmic reticulum (ER) stress markers (XBP-1s and GRP78) [33]. ER stress is involved in inducing EMT in different cell types, such as alveolar epithelial cells and thyroid epithelial cells [34] and plays a role in fibrotic remodeling during chronic inflammatory disease.

The treatment with PBA (4-phenylbutyric acid) or PP2 (c-Src kinase inhibitor) was demonstrated to be able to block the EMT induced by TGF- $\beta$ 1 via the c-Src pathway in primary nasal epithelial cells (PNECs) [33].

Recent studies have demonstrated a role for miR-21 in mediating TGF- $\beta$ 1-induced EMT in primary human nasal epithelial cells via the PTEN/Akt pathway during the pathogenesis of CRSwNP [35]. miR-21 inhibitors could be considered as anti-polyp drugs for treating nasal polyps (Li Xun, 2019) as well as recent findings suggest that glucocorticoids might prevent tissue remodeling by blocking the EMT initiated by TGF- $\beta$ 1-induced MAPK and Snail/Slug signaling pathways in CRSwNP [35,36].

### **1.3 SMAD3 and HIF-1 $\alpha$ signaling are involved in CRSwNP.**

Epithelial cells of nasal polyps showed an abnormal expression  $\alpha$ -SMA and when they were cultivated in hypoxia condition, EMT was induced via a SMAD3-dependent mechanism suggesting the crucial role for EMT in the pathogenesis of nasal polyps [37]. Shin et al demonstrated that hypoxia induced EMT independently of TGF- $\beta$ 1 signaling, by the suppression of PP2Ac (Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform) which is the catalytic subunit of protein phosphatase 2A implied in the dephosphorylation of phospho-Smad3 [37]. In nasal epithelium, EMT is driven cooperatively by Smad3 and HIF-1 $\alpha$ . Under hypoxia conditions hNECs expressed HIF-1 $\alpha$  and HIF-2 $\alpha$ : the first one mediates cytoskeletal rearrangement during hypoxia and the loss of E-Cadherin during EMT, the second protein could promote polyp growth by inducing cell proliferation. HIF-1 $\alpha$  inhibitors such as 2ME2 (methoxyestradiol) and 17-AAG (17-allylaminogeldanamycin) were found to suppress polypoid lesion development in a murine NP model opening the way to novel therapeutic strategies for nasal polyposis treatment [37].

### **1.4 WNT signaling is involved in CRSwNP.**

Wnt signaling dysregulation contributes to the impairment of epithelial function in CRSwNP. The up regulation of canonical Wnt signaling in CRSwNP results in an increase of  $\beta$ -catenin [38]. Although the mechanism is not completely elucidated,  $\beta$ -catenin accumulated in the cytosol moves to the nucleus where it cooperates to activate mesenchymal-related genes such as  $\alpha$ -smooth, muscle actin and vimentin.

The canonical WNT signaling activation by rhWNT3A or CHIR99021 (glycogen synthase kinase 3 inhibitor, a canonical Wnt agonist) treatment induced a significant increase of pro-inflammatory cytokines release in an *in vitro* model of normal HNEpCs define. Pro-inflammatory molecules are able to drive morphological changes in the epithelium, typical feature of remodeling in NPs [39].

Glycogen synthase kinase 3 is an important regulator of inflammatory processes involved in promoting the production of inflammatory cytokines (TNF, interleukin (IL-)1 $\beta$ , IL-6). A high expression of phosphorylated GSK-3 was detected in the nasal polyp tissue of patients with CRSwNP compared with the healthy mucosa [40]. Recent studies have shown that the monoterpene oxide 1,8-cineol is able to negatively modulate the Wnt/ $\beta$ -catenin signaling pathway by GSK-3 dephosphorylation in nasal polyps of chronic rhinosinusitis patients [41]. The presence of Wnt, the loss of E-cadherin, and increased  $\beta$ -catenin are important molecular parameters that define the EMT process.

### **1.5 PPAR $\gamma$ signaling pathway is involved in CRSwNP.**

PPAR $\gamma$  signaling pathway is also involved in EMT in CRSwNP. It has been shown that Rosiglitazone (ROG), a PPAR- $\gamma$  agonist, has an inhibitory effect on HMGB1 (High Mobility Group Box 1), a pro-inflammatory DNA-binding nuclear protein, inducing the epithelial cells to become mesenchymal-like cells and supporting the pathogenesis of eosinophilic chronic rhinosinusitis with nasal polyps ECRSwNP [42]. ROG reverted the effect of rhHMGB1 on EMT in ECRSwNP cells as well as the endogenous expression of HMGB1 induced by the treatment with the lipopolysaccharide (LPS). ROG is able to restore the effects of HMGB1 activation up-regulating the expression of ZO-1 (Zonula occludens-1) and E-cadherin and down-regulating the expression of N-cadherin and vimentin [42].

### **1.6 MEK1/2-ERK1/2 signaling pathways are involved in CRSwNP.**

The MEK1/2-ERK1/2 pathway is activated in CRSwNP [43]. There was an induction of the amount of MEK1/2 and phosphorylation of MEK1/2 and ERK1/2 in nasal polyps compared to healthy nasal mucosa. In CRSwNP patients MEK, pMEK, and ERK were localized primarily to the cells facing the basal membrane and scarcely in the upper layers of the epithelium and stroma. pERK was found in the nuclei of all of the cell layers in the epithelium of the polyps and was highly evident in the cells from the stroma of the turbinates of patients with CRSwNP. It appeared that ERK was activated in the epithelium of nasal polyps, associated with a role in acceleration of cell cycle. The ERK pathway could play a pivotal role in regulating the inflammatory process in CRSwNP in polyps and turbinates.

### **1.7 AGE/RAGE/ERK pathways are involved in CRSwNP.**

Recent studies demonstrated that AGE/RAGE/ERK pathway is involved in the pathogenesis of CRSwNP promoting EMT and tissue remodeling. The AGE/RAGE complex activated the ERK pathway sustaining trans differentiation of epithelial cells into mesenchymal cells and facilitating stromal tissue oedema formation and tissue remodeling [44].

The interaction between the products of non-enzymatic glycation and oxidation of proteins and lipids (AGE) with the Receptor of Advanced Glycosylation End products (RAGE) can be implied in the activation of several pathways including p38 Mitogen-Activated Protein Kinase (MAPK) and NF- $\kappa$ B [45] delineated in pelvic organ prolapse (POP) .

In the patients with neutrophilic chronic rhinosinusitis the ERK pathway is typically activated by high IFN- $\gamma$  expression. This activation correlated with an induction of markers of the EMT. IFN- $\gamma$  promoted the EMT in human nasal epithelial cells via both the JAK-STAT1-ICSBP-p38 as well as the ERK signaling pathways. The levels of expression of p-ERK and p-p38 increased with CRS progression in an independent-manner from the hypoxia-inducible factor (HIF-1 $\alpha$ ), SMAD, and NF- $\kappa$ B signaling pathways. A p38 inhibitor (SB203580) and a MEK inhibitor (PD98059) were

confirmed to be able to recapitulate the EMT hNECs phenotype [46]. Similarly, in a murine nasal polyp (NP) model, the number of NP lesions decreased after treatment with p38 and ERK inhibitors as well as the secretion of neutrophils but not eosinophils. The targeting of p38 and ERK signaling pathways is proposed to be a novel therapeutic strategy against neutrophil-dominant CRS [46].

### **1.8 Intelectin-1, Proconvertase 1 and Sirtuin 1 are dysregulated in nasal polyps.**

In addition to alterations in cellular signaling pathways, concentrations of specific proteins are dysregulated in nasal polyps compared to normal control nasal tissue, such as Intelectin-1, Proconvertase 1 and Sirtuin 1. Intelectin-1 is a microbial galactofuranose-binding lectin, playing a role in immune defense against bacteria. Intelectin-1 is normally expressed in healthy sphenoid sinus mucosa, and is increased in patients with nasal polyps [47]. The exact reason underlying the overproduction of intelectin-1 in nasal polyps is not known; it is conceivable that this antimicrobial peptide could sustain chronic inflammation by increasing interleukin-13-mediated chemokines production and the monocyte chemoattractant protein-1 and -3 [48].

Basal epithelial cells in CRS express high levels of the hormone-processing enzyme proconvertase 1 (PC1/3), selectively expressed in neuroendocrine cells. In nasal polyps, PC1/3 expression was positively correlated with loss of E-cadherin expression and gain in expression of N-cadherin, collagen I and MMP-2. PC1/3 overexpression could sustain biochemical and morphological changes in EMT of airway epithelial cells contributing to the pathogenesis of nasal polyps [49].

Sirtuin1 (SIRT1) protein was instead downregulated in the mucosa cells from patients with nasal polyps compared with the levels observed in the cells from patients without polyps. It was demonstrated that SIRT1 overexpression or activation is able to reverse hypoxia-induced EMT in human nasal epithelial cells possibly because of inhibition of HIF-1-induced EMT [50]. SIRT1 exerts its activity by the deacetylation of acetylated lysine by hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) implicating Sirtuin 1 as a potential therapeutic target in nasal polyp treatment [50]. Resveratrol is able to activate SIRT1 preventing development of eosinophilic rhinosinusitis with nasal polyps in a mouse model [51] especially when it conjugated with a cell penetrating peptide (CPP) [52].

## **2. Nasal Polyp-derived Mesenchymal Stem Cells**

Mesenchymal stem cells are multipotent stromal cells that are present in multiple tissues, including bone marrow, fat tissue and umbilical cord. Mesenchymal stem cells are able to self-renew and have the potential to differentiate into adipocytes, osteoblasts, myocytes and chondrocytes *in vivo* and *in vitro* [53,54]. Under specific culture conditions, MSCs can differentiate into non-mesodermal lineages such as hepatocytes, neurons, pancreatic cells, cardiac muscle cells or astrocytes [55].

Nasal polyp tissue has also been explored as a novel source of MSCs maintaining the stemness features and differentiation potential following multiple rounds of passaging [26]. Nasal polyp-derived MSCs are usually isolated from polyp tissues by mechanical separation followed by enzymatic digestion in collagenase IV for 1h at 37°C. The reaction is inactivated by medium complete with serum then MSCs are plated and cultivated. After a short lag period, polyp derived MSCs become plastic-adherent and show spindle shaped morphology according to the indications of Friendstain A.J. [56-58].

The PO-MSCs phenotype is similar to that of MSCs derived from bone marrow or adipose tissue and is characterized by a negative expression for hematopoietic surface markers (CD34, CD45 and HLA-DR) and a positive expression for classical mesenchymal surface antigens, CD105, CD44, CD54, CD90, and CD73 [26,57,59]. The PO-MSCs show high clonogenic abilities and can be passaged up to 15 times maintaining their self-renewal ability [57]. These PO-MSCs are adult multipotent stromal stems cells, able to differentiate into several different classical mesenchymal derived cell types, osteocytes, adipocytes and chondrocytes as well as having ability with the appropriate stimulus to form neuron like cells [57,59-61].



Initially PO-MSCs have a fibroblastoid appearance, after osteogenic induction take on a cuboidal shape and the deposition of calcium salt nodules becomes appreciable [57]. Osteogenic lineage commitment is supported by the expression of osteoblast-specific genes as RUNX2, the osteogenic master regulator, and osteocalcin, a late marker for osteoblastic maturation [62]. Osteogenic differentiation can be obtained from MSC cells derived from nasal turbinate (TMSCs) as well as nasal septal deviation, with increased gene expression of BSP, Runx2, BMP2, OSX, and Col1 [63].

When the PO-MSCs are grown in an adipogenic induction medium for 21 days some cells showed a tendency to form spherical accumulations of multiple intra-cellular lipid filled droplets [57,59] which can be detected by Oil Red O staining. These PO-MSCs express an increased gene expression of the PPAR $\gamma$  a key player in controlling the transcriptional pathway of adipogenesis, as well as the target gene FABP4 [57] and the transcription factor ZNF423 as found in adipocytes derived from mesenchymal stem cells [64,65]. In addition, recent evidence suggest that the fine balance between some zinc finger proteins, such as ZNF521/ZNF423, is relevant for maintenance of stemness in mesenchymal stem and progenitor cells [64,66-67].

PO-MSCs are also able to generate chondrocyte like cells *in vitro* PO-MSCs cells induced with the chondrogenic medium acquired a rounded and enlarged morphology and expressed the chondrogenic differentiation markers Sox9 and Col2A [57,68]. Sox9 is a transcription factor involved in cartilage formation and exerts its function as activator of type II collagen, the main component of cartilage [69].

PO-MSCs can differentiate *in vitro* into cells of non-mesodermal origin, such as neuron-like cells. Jung-Sun Cho, 2015, displaying neurofilament heavy chain (NF-H), and when cultured as xenogeneic co-culture with sliced adult rat brain biopsy neurofilament, nestin and GM-CSF could be detected [60]. Delorme et al. and Girard et al., [61,70], showed that olfactory ectomesenchymal stem cells (OE-MSCs) which originate from a neural crest-derived tissue could differentiate towards osteocytes as well as neuronal like cells when stimulated for neural differentiation an increased expression of neural cell-related proteins including  $\beta$ -tubulin III, Nestin, GFAP, O4 and MAP2 were detected. These cells had a relative disinclination to give rise to chondrocytes or adipocytes compared to classical MSCs sources.

Systematic studies are required to determine the relative ability of nasal polyps to form MSCs and differentiate into the different types of cells compared to normal nasal tissue from different parts of the nose as either healthy adjacent biopsy or of control normal subjects. Comparisons with other sources of MSCs from the bone marrow, adipose tissue, umbilical cord and dental pulp would be useful to appreciate the relative abilities for each differentiation. Different MSCs will have an inherent complement of suppressing and activating transcription factors, which will determine the degree of response for each type of differentiation stimulus. Currently there are available commercial differentiation cocktails which should permit standardization of protocols.

### 3. Gene expression studies on nasal polyps

To identify the molecular properties of PO-MSCs, de Oliveira et al. [59] carried out a global gene expression profile of PO-MSCs in comparison with BM-MSCs. Comparing 4 samples of each, 15 genes were significantly upregulated including PROM1 or CD133, a stemness marker typical of haematopoietic stem cells, and ABCB1 (ATP-binding cassette sub-family B member 1) a protein expressed in human fetal neural stem/progenitor cells at an early developmental stage [71]. Hepatocyte nuclear factor 1-alpha (HNF1) gene also had a fold-change index significantly higher compared to BM-MSCs. HNF1 is a transcriptional activator required for the expression of several human embryonic stem cell specific genes involved in cell growth, cell adhesion, epithelial formation, immune system, and inflammation.

This evidence supports the idea that PO-MSCs have a distinct individual molecular profile that appears in part different from BM-MSCs. For example, POU2F1 and TFAP4 genes transcriptional regulators involved in cancer stem cells and cell cycle were upregulated compared to BM-MSCs [59].

In contrast PO-MSCs showed a reduced expression of cytokines and growth factors (GDF6, KDR, FGF10, and GDF5) when compared to BM-MSCs [59]. Despite fact that PO-MSCs share many important characteristics with BM-MSCs including the cellular phenotype and the multi lineage potential, they also show different immune regulatory profiles. Immune-associated molecules (CD117, HLA-DR, PDL-1, and PDL-2) are lost in PO-MSCs, resulting in a reduction of immunoregulatory abilities such as the inhibition of lymphocyte proliferation and the regulatory T cell expansion [59].

Gene expression of the transcription factors T-bet, GATA3, RORC and FOXP3 were evaluated in a set of 14 CRSwNP and 8 CRSsNP samples [72] which revealed that eosinophilic CRSwNP was characterized by higher level of GATA3 gene expression compared to noneosinophilic CRSwNP. Tbet, GATA3, RORC were higher in CRSsNP than CRSwNP whereas there was little difference for the FOXP3 gene. The expression of RORC implicates an involvement of the nasal immune response [72] better preserved in the CRSsNP patients than those with polyps.

Next generation sequencing (NGS) [73], has been used to compare CRSwNP and controls using a bioinformatic approach based on data from Plager et al. [74] and Stankovic et al. [75]. NGS profiling represents a non biased methodology to identify gene and pathway changes. The analysis gave a total of 538 DEGs (326 up-regulated and 212 down-regulated) with enrichment for hematopoietic cell lineage and salivary secretion pathways. Modules were also identified, which were highly associated with chemokine signaling pathways, Th1 and Th2 cell differentiation.

CRSwNP compared to normal control nasal tissue samples were used to obtain transcriptome profiles of mRNAs and long non-coding RNAs (lncRNAs) [76,77]. 265 differentially expressed lncRNAs and 994 mRNAs were identified mostly associated with signal transduction. Enriched pathways included cytokine-cytokine receptor interactions and cell adhesion molecules. lncRNAs were identified, which regulate chemokine (C-C motif) ligand 18 (CCL18), inflammation and polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7) for cell proliferation. These genomic data provide a foundation for future investigations into mRNAs and lncRNAs as diagnostic and therapeutic targets in CRSwNP.

#### 4. Microenvironmental factors affected CRSwNP

The nasal microenvironment is also critically involved in nasal polyposis and progression. Bone marrow-derived MSCs were found to modulate the cell phenotype in the nasal polyp microenvironment. When BM-MSCs were co-cultured with nasal polyp-derived cells, cultures exhibited a direct immunomodulation on the inflammatory polyposis that resulted in a significant increase in CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells and a significant decrease in the frequency of CD4<sup>+</sup>, CD8<sup>+</sup>, CD14<sup>+</sup>, and NK cells, and finally promoted a strong inhibition of CD4<sup>+</sup> and CD8<sup>+</sup> T cell proliferation [78]. In addition, in co-culture conditions, the immunoregulatory effects were associated to a change of the global cytokine profiles with an increase in anti-inflammatory molecules such as IL-10 and a decrease in inflammatory cytokines such as IL-2, TNF- $\alpha$ , and IFN- $\gamma$  [78].

In other co-culture experiments [79] mouse adipose-derived stem cells (ASCs) exerted immunomodulatory effects in eosinophilic NP consisting in a down-regulation of Th2 cytokines and in an up-regulation of Th1 and regulatory cytokines; however the number of T lymphocytes was unchanged compared to control mucosa.

#### 5. Conclusions

Chronic rhinosinusitis with nasal polyps (CRSwNP) is one of the most common respiratory disease worldwide. This disorder affects over 10% of the adult population and the prevalence increases with age, causing a significant reduction in patients' quality of life. Although the molecular pathogenesis of CRSwNP is not completely clear, EMT has been identified to play a central role in the nasal tissue remodeling and persistent inflammatory process. At present, the treatment options for CRS include the use of oral antihistamines to relieve symptoms of allergies, antibiotics to cure the

chronic or recurring infection, topical steroids to reduce the inflammation, as well as humanized antibodies targeting either circulating IL-5 or its receptor expressed on eosinophils and basophils which are able to exert a potent neutralizing activity. The recurrence of polyps and symptoms occurs very frequently in patients with CRSwNP even after pharmacological and surgical treatment. For this reason, the investigation of signaling pathways associated with EMT from CRSwNP is a crucial step in identifying new therapeutic targets. Targeting EMT-related signal pathways could have an impact on reducing the rate of CRS offering an attractive therapeutic strategy in the treatment of patients with CRSwNP.

In addition, nasal polyps represent an alternative source of MSCs (PO-MSCs) having similar features found in BM-MSCs. Nasal polyp derived mesenchymal stem/progenitor cells are an amenable model for *in vitro* investigation for molecular mechanisms underlying the inflammatory process responsible of nasal tissue remodeling. The PO-MSCs, because of their immunomodulatory properties, could be represent a promising treatment for several human diseases and in the future could be used for the development of regenerative therapies. The development of suitable animal models for CRSwNP will aid this approach.

Further studies are needed to clarify the differentiative potential of PO-MSCs, the mechanism of action in lesions of the nasal polyps and their possible application for the development of regenerative therapies.



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## Abbreviations

CRS	Chronic rhinosinusitis
EMT	Epithelial-mesenchymal transition
PO-MSCs	Polyps derived mesenchymal stem cells
CRSwNP	CRS with nasal polyps
CRSsNP	CRS without nasal polyps
ECP	Eosinophilic cationic protein
EDN	Epithelium-derived neurotoxin
$\alpha$ -SMA	alpha-smooth muscle actin
MMP	Matrix metalloproteinases
TGF- $\beta$	Transforming growth factor beta
XBP1	X-box binding protein 1
GRP	Glucose-regulated protein 78
PNECs	Primary nasal epithelial cells
PP2Ac	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform
2ME2	Methoxyestradiol
17-AAG	17-allylaminogeldanamycin
HNEpCS	Human Nasal Epithelial Cells
GSK-3	Glycogen synthase kinase 3
ROG	Rosiglitazone
LPS	Lipopolysaccharide
HMGB1	High mobility group box 1 protein
ZO-1	Zonula occludens-1
AGE	Advanced Glycation Endproducts
RAGE	Receptor for advanced glycation endproducts
NF- $\kappa$ B	Nuclear factor- $\kappa$ B
HIF-1 $\alpha$	hypoxia-inducible factor
PC1/3	Proconvertase 1
SIRT1	Sirtuin1
CPP	Cell Penetrating Peptide
RUNX2	Runt-related transcription factor 2
TMSCs	MSC cells derived from nasal turbinate
BSP	Bone sialoprotein
BMP2	Bone morphogenetic protein-2
OSX	Osterix
Col1	type I collagen
PPAR $\gamma$	Peroxisome proliferator-activated receptor gamma
FABP4	Fatty acid-binding protein 4
Sox9	SRY-Box Transcription Factor 9
Col2A	type IIA collagen
OE-MSCs	Olfactory Ectomesenchymal stem cells
GFAP	Glial fibrillary acidic protein

MAP2	Mutual protection of microtubule-associated protein 2
BM-MSCs	Bone marrow mesenchymal stem cells
PROM1	Prominin-1
ABCB1	ATP-binding cassette sub-family B member 1
HNF1	Hepatocyte nuclear factor 1-alpha
POU2F1	POU Class 2 Homeobox 1
TFAP4	Transcription Factor AP-4
GDF6	Growth Differentiation Factor 6
KDR	Kinase Insert Domain Receptor
FGF10	Fibroblast Growth Factor 10
GDF5	Growth/differentiation factor 5
HLA-DR	Major Histocompatibility Complex, Class II, DR Alpha
PDL-1	Programmed death-ligand 1
PDL-2	Programmed death-ligand 2
RORC	RAR Related Orphan Receptor C
FOXP3 -2	Forkhead box protein 3-2
TNF- $\alpha$	Tumor necrosis factor alpha
IFN- $\gamma$	Interferon gamma

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